SYNOPSIS OF P. G. RESEARCH PROBLEM

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3.	**Faculty**	:	Agriculture
4.	**Name of the Degree Submitted for**	:	Ph.D. (Agri.)
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8.	**Title of research problem**	:	**GENETIC ARCHITECTURE AND MOLECULAR CHARACTERIZATION OF SEED YIELD AND YIELD COMPONENTS OF PIGEONPEA (*Cajanus cajan* (L.) Millsp.)**

9. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.], is an often cross pollinated pulse crop belonging to the family fabaceae with a diploid chromosome number of 2n=2x=22, having a genomic size of 833.07 Mb. It is also known by common names such as Arhar, tur and red gram. It contains protein content of 18-24 % per 100g of whole seed or split grain, and also provides carbohydrates, fiber, minerals and vitamins. Additionally, it it has a rich composition of essential amino acids of lysine, cysteine, tyrosine and arginine, as well as good composition of iron and iodine. Due to these nutritional benefits, it is considered as one of the major dietary pulse crops in India.

India holds a dominant position in the global pigeonpea, ranking first in both area and production. Pigeonpea in india grown in an area of 4.33 lakh ha with a production of 3.6 million tonnes with a productivity of 827 kg/ha. The highest production is in Maharashtra, followed by Karnataka, Jharkhand and Gujarat. According to 2024-25 estimates of kharif pigeonpea in Gujarat, the area sown under was 1.73 lakh ha, and production was 2.01 lakh tonnes with a productivity of 1162 kg/ha (Indiastat- MoAF&W, GoI.).

India is regarded as the primary origin of the pigeonpea due to its widespread presence of wild relatives and wild forms having high genetic variability. (van der

Maesen, 1990). It is able to withstand drought and other abiotic stress. It is also called as a biological plough as its tap root system spreads and breaks up the hardy soil. Pigeonpea is one of the crops that is more suitable for dry-land farming and intercropping with other crops to enhance the soil fertility gradient and productivity. They have root nodules that have a symbiotic association with rhizobium bacteria, which helps in fixing the atmospheric nitrogen, thereby restoring and maintaining the soil fertility. However, the restricted availability of genetic variability has led to production remaining more or less the same over time (Yang *et al*., 2006). So, there is a need to improve the genetic potential in the cultivated breeding material to enhance the yield via diverse breeding programmes.

The genetic enhancement of crop varieties for commercial quality parameters through intraspecific and interspecific hybridization can be utilized to develop varieties for disease and pest resistance, and it also helps in broadening the genetic base. (Stebbins, 1958). Many researchers have put their efforts into overcoming the yield plateau and it can be achieved through developing heterotic hybrids. By utilizing the concept of heterosis, there has been a positive change in the yield (Chuadhari *et al.*, 2025).

As the breeding techniques are advancing and their evaluation through new methods is helping in the development of new varieties. This involves working on the stability of genotypes, estimating parents for their combining ability, and identifying superior hybrid combinations by utilizing combining ability and gene action. Understanding these factors, along with their diversity association at the molecular level, is highly beneficial for successful variety development.

10. Practical Utility of the Research Project

Heterosis refers to a hybrid's superior performance over its parents in yield, quality, disease resistance, or other morphological, physiological, or biochemical characteristics and the term coined by Shull in 1914, which is typically divided into relative heterosis, heterobeltiosis, and standard heterosis; plant breeders consider standard heterosis, measured against a standard check is the most practically valuable. The level of heterosis depends on how genetically divergent the parent lines are and on the extent of dominance. To achieve maximum heterosis, the inbred lines chosen for crossing should have completely contrasting allele frequencies.

Combining ability is the most effective tool for identifying suitable parents for hybridization. Selecting parents solely based on their per se performance may not

always yield fruitful results (Allard, 1960). Studies on the general combining ability (GCA) of parents and the specific combining ability (SCA) of hybrids help in selecting appropriate parents and hybrids for an efficient breeding programme. The diallel mating method is considered the best approach for evaluating the combining ability of parents (GCA) and hybrids (SCA). Griffing (1956) developed the statistical analysis of data from diallel mating designs to estimate GCA and SCA variances and effects.

Several researchers contributed to the development of the diallel mating design concept (Hayman, 1954a, 1954b; Griffing, 1956). Understanding the mode of gene action that influences seed yield and its associated components, is crucial for developing appropriate breeding strategies for polygenic traits. Pigeonpea yield can be enhanced by exploiting heterosis and hybrid vigour as well as significant additive and non-additive gene action (Yamanura *et al.*, 2016). The general combining ability variance provides an estimate of additive variance, while the specific combining ability variance provides an estimate of dominance variance. The ratio of GCA to SCA variance gives an indication of the type of gene action governing the quantitative character, i.e., additive or non-additive. The average degree of dominance provides an estimate of the degree of dominance governing the trait, i.e., complete dominance, incomplete dominance, or overdominance.

In any breeding programme, it is necessary to identify phenotypically stable genotypes that perform more or less uniformly across diverse environments. Stability refers to a varietal ability to maintain a consistent yield under varying environmental conditions, as measured by various stability parameters. The phenotype of an individual is determined by G × E interaction. Genotypes show different levels of phenotypic expression under different environmental conditions, resulting in varied performances; in such cases, the genotype × location interaction is an important factor in plant breeding strategies. The AMMI model (Gauch, 1992) handles this by using ANOVA for additive effects and IPCA for the multiplicative part (Ojwang *et al.*, 2021; Bomma *et al.*, 2024). AMMI biplots plot genotypes and environments on the same axes to show their relationships. AMMI stability values (ASV) rank genotypes by stability (Bomma *et al.*, 2024), and the yield stability index (YSI), which combines mean yield with stability, helps select genotypes that are both stable and high-yielding (Kang, 1993).

The success of any breeding effort depends on the amount of variability present for the traits of interest. Variability is essential for resistance to biotic and abiotic stresses and for broad adaptability; selection works only when genetic differences exist

among individuals. Thus, knowing the extent of genetic variability in a population is critical for planning an effective breeding program. Over the years, methods for detecting and assessing genetic diversity have been improved through advances in molecular markers. DNA-based markers, enabled by PCR, have transformed molecular biology. Among them, microsatellites (simple sequence repeats, SSRs) are particularly suited for estimating genetic diversity and identifying varieties. SSRs are short 1–6 bp tandem repeats spread throughout the genome, forming a PCR-based, multi-allelic, co-dominant marker system. They are highly abundant, hypervariable, co-dominant, inherit in a Mendelian fashion, and are technically simple and sensitive, making them ideal for genetic diversity analysis, and germplasm diversity studies (Ma *et al.*, 2011; Zhang *et al.*, 2007; Zhou *et al.*, 2003), including heterosis applications, especially for closely related species. Researchers have used SSRs to study pigeonpea diversity (Kumar *et al.*, 2023; Reddy *et al.*, 2022; Sharma *et al.*, 2020). This work offers useful insights for strengthening pigeonpea improvement efforts.

11. Objectives

By keeping this in view, the present investigation on "Genetic architecture and molecular characterization of seed yield and yield components of pigeonpea (*Cajanus cajan* (L.) Millsp.)" will be undertaken with the following objectives:

1. To estimate the magnitude of heterosis for seed yield and its contributing traits.
2. To estimate the general combining ability for crosses and specific combining ability to crosses for seed yield and its contributing traits.
3. To compare nature and magnitude of gene action involved in the inheritance of seed yield and its contributing traits.
4. To estimate genotype × environment interaction and phenotypic stability analysis by using AMMI model for seed yield and its contributing traits.
5. To characterize pigeonpea genotypes using SSR markers.

12. Review of Literature

The literature pertaining to the heterosis, combining ability analysis, gene action, stability and parental diversity for seed yield and its component traits in pigeonpea has been reviewed under the following subheads.

12.1 Heterosis

Bhanu *et al*. (2017) evaluated four pigeonpea crosses—Bahar × IPA 204, Bahar × BSMR 846, MAL 13 × BSMR 846, and MA 6 × BDN 2029—to assess heterosis for

grain yield and its component traits in comparison with mid-parent, better-parent, and standard variety values. Among these, the cross MAL 13 × BSMR 846 consistently performed the best, exhibiting the highest positive mid-parent heterosis for seed yield per plant, with Bahar × BSMR 846 ranking next. A similar trend was observed for pods per plant, where MAL 13 × BSMR 846 again recorded the maximum mid-parent heterosis, followed by Bahar × BSMR 846. When compared to the standard variety, MAL 13 × BSMR 846 once more showed superior performance for seed yield per plant, while MA 6 × BDN 2029 followed it. Notably, MAL 13 × BSMR 846 also registered the highest heterosis for pods per plant over the standard variety.

Kumar *et al*. (2017) assessed 28 pigeonpea hybrids derived from an 8 × 8 diallel mating design for evaluating heterosis. Among these, the crosses UPAS-120 × GT-103, BSMR-853 × Banas, and BSMR-853 × GT-1 showed significant positive heterosis over the mid-parent. Interestingly, BSMR-853 × Banas and BSMR-853 × GT-1 also maintained significant superiority when compared to the better parent. When performance was assessed against the standard check, ICPL-87119 × GT-103 (13.75%) and ICPL-87119 × AGT-2 (13.48%) recorded the highest significant positive heterosis. Considering both their heterotic response and overall per se performance, these two crosses were identified as the most promising among the evaluated hybrids.

Anusha *et al.* (2019) evaluated 33 pigeonpea genotypes, including 7 CMS (A) lines, 9 testers (R-lines), 16 hybrids, along with a check variety, to estimate the extent of heterosis. The analysis showed that seed yield per plant exhibited significant relative heterosis in five hybrids, with values ranging from –23.51% to 70.31%. In comparison, only two hybrids displayed significant heterobeltiosis, varying between –39.87% and 36.88%, while four hybrids showed significant heterosis over the standard check, ranging from –51.03% to 28.99%. When overall performance was considered, particularly for seed yield and its contributing traits, hybrids such as ICPH 3481, ICPH 3496, ICPH 2438, and ICPH 2363 stood out. These crosses were identified as the most promising, as each recorded more than 25% standard heterosis for seed yield along with key yield-attributing characters.

Patel *et al.* (2020) evaluated 14 pigeonpea parents to determine the magnitude of heterosis that was mated in line × tester fashion. The ANOVA revealed that the mean sum of squares was significant for all traits, both when comparing parents with hybrids

and among the hybrids themselves. Taking these into account per se performance along with different measures of heterosis, namely standard, mid-parent, and better-parent, the hybrids CMS GT 1603 A × GTR 23, CMS GT 288 A × GTR 55, CMS GT 1603 A × GTR 97, CMS GT 1616 A × GTR 55, and CMS GT 1616 A × GTR 97 were identified as the most promising combinations.

Tiwari *et al.* (2022) analysed 12 parental lines and 35 F_1 hybrids of pigeonpea developed using a Line × Tester design to understand heterosis for seed yield per plant. Interestingly, all the crosses showed positive mid-parent heterosis for this trait. Among them, a few hybrids stood out with significant positive mid-parent heterosis, including ICP7376 × ICPL 87119, ICP7376 × ICPL-87, ICP7382 × UPAS-120, ICP7406 × UPAS-120, ICP7382 × ICPL-87, ICP7004 × JKM-189, ICP7406 × JKM-189, and ICP7406 × ICPL 87119 (12.26%). When compared to the better parent, only two crosses, ICP7382 × UPAS-120 and ICP7004 × JKM-189 showed considerable positive response of for seed yield per plant.

Bisht *et al.* (2023) investigated heterosis in pigeonpea by crossing eight genotypes in a half diallel manner to produce 28 F1s. The hybrids Pusa 992 × Pant A 441, UPAS 120 × Pant A 441 and UPAS 120 × Pusa 992 emerged as the top performers for seed yield, with Pant A 441 serving as a valuable donor for high seed yield. The findings indicated that heterosis breeding could be effectively utilized to enhance seed yield per plant along with its associated traits in pigeonpea. Among the hybrids evaluated, Pant A 441 × AH 09-47 and Pant A 441 × Pusa 2013-2 stood out by exhibiting high standard heterosis for seed yield.

Savaliya *et al.* (2023) evaluated 30 CGMS using a line × tester design, pigeonpea hybrids were developed by crossing four CMS lines with seven restorer lines to evaluate heterosis. The ANOVA revealed that the average sum of squares due to genotypes was significant for all the traits studied, indicating that the parental lines involved in the crosses had adequate genetic variability. Five specific cross combinations, GNP 3A × GNPR-20-18, GNP 3A × GNPR-20-10, GNP 4A × GNPR-21-23, GNP 1A × GNPR-20-18, and GNP 4A × GNPR-21-29, exhibited significant positive heterobeltiosis as well as significant standard heterosis relative to the check varieties GT 104 and GTH 1.

Chuadhari *et al.* (2025) investigated 6 genotypes of pigeonpea in half diallel mating fashion. Across the evaluated crosses, favourable heterobeltiosis was most commonly observed for traits such as pod length, followed by pods per plant, days to maturity, plant height, primary branches per plant, and days to 50% flowering. It was also evident for secondary branches per plant and seed yield per plant, although to a relatively lesser extent compared to the other traits. On the other hand, traits like seeds per pod, 100-seed weight, and protein content showed favourable heterobeltiosis in comparatively fewer crosses.The hybrid combinations BP-16-184 × NP-09-30, UPAS-120 × NP-09-30, NP-09-26 × NP-09-30, and BDN-711 × NP-09-26 emerged as the most heterotic for seed yield per plant and other yield related traits.

Sharma *et al.* (2025) evaluated eight parental genotypes of pigeonpea using a half-diallel mating design. For pigeonpea yield per plant, the three hybrids ICPL-20338 × ICPL-20340, ICPL-20340 × AL-882, and ICPL-20338 × AL-882 performed best based on their per se performance and standard heterosis, and they also excelled for yield-related traits such as pod number per plant, seeds per pod, biological yield per plant, and 100-seed weight.

Koli *et al.* (2025) assessed 12 parental lines (8 pure lines and 4 testers) together with their 32 F_1 cross combinations, plus the commercial hybrid ICPL-87119. Hybrids RKPV-702-16 × ICPL-88039, RKPV-705 × ICPL-88039, RKPV-807 × PA-291, RKPV-808 × UPAS-120 and RKPV-821-01 × ICPL-88039 recorded substantial heterosis in a positive direction for yield per plant across all evaluation methods. The significant positive standard heterosis observed for seed yield per plant.

2. Combining ability and gene action

Parmar *et al.* (2012) In their study, Parmar et al. (2012) assessed 12 CGMS lines crossed with five restorer lines through a line × tester mating design. The combining ability analysis indicated that non-additive gene action predominated across all the traits studied, emphasising the potential of heterosis breeding for improving seed yield and its associated components in pigeonpea. Among the parental lines, ICPA 2086, ICPA 2089, ICPA 2052, ASHA, and GT 101 emerged as strong general combiners for seed yield. In terms of specific combining ability, the crosses ICPA 2086 × ASHA, ICPA 20 × JKE 114, and GT 33 × JKM 189 showed superior SCA effects for seed yield per plant.

Patel *et al.* (2013) carried out a diallel analysis using seven pigeonpea genotypes to understand the nature of gene action and the extent of combining ability for grain yield per plant and its related traits. The results revealed that both additive and dominance gene effects played a role in the expression of all the characters studied. However, since the GCA variance was consistently lower than the SCA variance for each trait, dominance effects were found to be more prominent. Among the parents, ICPL-87119, AGT-2, and GT-102 were identified as efficient general combiners for yield per plant, pods per plant, seeds per pod, and 100-seed weight. In terms of cross performance, GT-102 × ICPL-87119, BSMR-853 × GT-102, and AGT-2 × GTR-11 emerged as the most promising combinations for seed yield per plant.

Yamanura *et al.* (2014) carried out an experiment eight cytoplasmic-genetic male sterile (CGMS) lines possessing A2 cytoplasm from Cajanus scarabaeoides and A4 cytoplasm from Cajanus cajanifolius, which were crossed with 10 testers in a line × tester design to generate 80 hybrids. The combining ability analysis of these hybrids revealed that non-additive gene action predominantly governed all ten traits studied, highlighting the usefulness of heterosis breeding for improving yield.With respect to general combining ability, the parents ICPA-2078, GT-308A, PKV-TATA, ARCCV-2, and GPHR-08-11 were identified as superior combiners for seed yield and its major components. In terms of specific combining ability, nine hybrids exhibited significant positive SCA effects for seed yield. Among these, ICPA-2092 × VIPUL, ICPA-2078 × BSMR-856, and ICPA-2078 × ARCCV-2 stood out as the best specific combinations for traits such as number of secondary branches, pods per plant, and seed yield per plant.

Yamanura *et al.* (2016) evaluated three CGMS lines which were mated with 17 testers following a line × tester design, producing ample hand-pollinated seed. Combining ability analysis indicated that non-additive gene action predominated for seven traits, highlighting the importance of heterosis breeding for yield improvement.For seed yield and its related traits, the parents ICPA-2043, GRG-2009, GRG-2009-2, LAXMI, LRG-41, and JKM-197 emerged as strong general combiners, indicating good GCA effects. The specific combining ability analysis further identified 11 hybrids that exhibited significant, positive, and favourable SCA effects for seed yield per plant. Among these, the crosses ICPA-2043 × GRG-2009-2, ICPA-2047 ×

GRG-2009, and ICPA-2043 × ICPL-288 were selected as the most promising based on their superior per se performance. Notably, these combinations also recorded high mean values along with significant positive SCA effects, which were associated with high × high GCA effects of their respective parents.

Moses *et al.* (2020) evaluated combining ability in a set of 12 crosses developed by mating four lines with three testers using a line × tester design. The analysis of variance indicated the presence of highly significant differences among lines and testers for all traits studied, except for the number of primary branches. Among the parents, the lines BRG-2 and B2-10 along with the testers BRG-1 and B1-169-1 exhibited significant GCA effects, identifying them as efficient general combiners for seed yield and its associated traits. Based on specific combining ability performance, several hybrids were recognized as promising, including BRG-2 × BRG-1, B2-10 × BRG-1, B3-13 × BRG-1, B2-5-2-1 × B1-169-1, BRG-2 × BRG-3, B2-10 × B1-169-1, and BRG-2 × BI-169-1.

Patel *et al.* (2020) carried out a combining ability study on 40 pigeonpea genotypes using a line × tester design. The analysis showed that the ratio of GCA to SCA variance was less than unity for all traits except days to maturity, indicating the predominance of non-additive gene action. Among the parental lines, CMS GT 1603 A, CMS GT 1616 A, CMS GT 288 A, CMS GT 307 A, and GTR 97 were identified as effective general combiners for seed yield and its contributing traits, suggesting their usefulness as valuable genetic resources. In terms of specific combining ability, 12 hybrids exhibited significant positive SCA effects for seed yield, with CMS GT 288 A × GTR 55, CMS GT 1616 A × GTR 55, CMS GT 1002 A × GTR 97, CMS GT 1402 A × GTR 18, and CMS GT 1602 A × GTR 23 emerging as the top performers. Considering both mean performance and SCA effects, the hybrid CMS GT 288 A × GTR 55 stood out as the most promising and may be prioritized in future pigeonpea improvement programmes.

Bisht *et al.* (2022) evaluated 28 F_1 progeny of pigeonpea derived from crosses among 8 parents arranged in a half-diallel design. The analysis of variance indicated that both additive and dominance gene actions played a role in the expression of the studied traits; however, the SCA variance was consistently higher than the GCA variance, suggesting a stronger influence of non-additive effects. Among the parents,

Pant A 441, Pusa 992, and AH 09-471 were identified as efficient general combiners, showing significant GCA effects for yield and its associated traits. Based on overall performance, the cross Pant A 441 × Pusa 2013-2 stood out as the most promising, with the potential to produce desirable transgressive segregants in the subsequent generations.

Gaur *et al.* (2022) evaluated the combining abilities of 45 pigeonpea hybrids along with their 10 parental lines. Among these 42 heterotic crosses, 21 displayed favourable SCA, 13 displayed unfavourable SCA, and 8 displayed intermediate SCA effects. The highest positive mean GCA of both parents (MGCA) was observed in Pusa 992 × PA 622, followed by PA 624 × PA 622 and Paras × PA 622. The mean GCA values were notably positively and correlated significantly with mid-parent heterosis, better-parent heterosis, and standard heterosis.

Jayamala *et al.* (2023) assessed 13 pigeonpea genotypes for evaluating combining ability using a line × tester design. The results indicated that MS Prabhat DT and CORG 9060 were efficient general combiners for traits related to early flowering and maturity. On the other hand, MS Co 5 and CORG 5 exhibited strong general combining ability for plant height, branching, number of pods, and grain yield per plant. In terms of cross performance, MS Co 5 × ICPL 161 and MS Co 5 × CORG 5 emerged as the most promising combinations for grain yield. Additionally, MS T 21 × CORG 9060 showed high SCA effects for branches per plant, while MS T 21 × ICPL 8801 recorded desirable negative SCA effects for days to first flowering and maturity. The crosses MS Co 5 × ICPL 161 and MS Co 5 × CORG 5 also exhibited high SCA values for seed yield per plant. Notably, these hybrids involved parents with good GCA and expressed heterosis over the better parent, highlighting their breeding potential.

Vardhini *et al*. (2024) analyzed 24 pigeonpea hybrids, developed through the line × tester method. Among the parental lines, GJP 1915 showed strong general combining ability for seed yield per plant, along with related traits such as plant height, primary branches, and pod length. Likewise, the tester GJP 1820 also exhibited good GCA for seed yield per plant, as well as for plant height, primary and secondary branches, pods per plant, and 100-seed weight. For specific combining ability, the cross BDN 2013-41 × UPAS 120 recorded the highest and most significant SCA effect for seed yield per plant. Another promising hybrid, GJP 1915 × BLACKTUR, also showed

a significant positive SCA effect for seed yield per plant and performed well for pod length. Overall, these crosses appear useful for future breeding efforts aimed at exploiting hybrid vigor.

3. Genotype x Environment interaction and phenotypic stability analysis

Singh *et al.* (2018) experimented with 21 genotypes of pigeonpea to analyse the genotype x environment interaction by using AMMI stability analysis. The first principal component (PCA1) of the interaction explained more than 60% of the interaction variation across nearly every trait examined. Average seed yield per plant peaked at 39.15 g and was statistically similar across all three years. Nine genotypes, PUSA 2003-1, CORG-2001-5, WREG-28, PANT-A-286, H-94-6, GT 101, ICPL-99004, ICPL-85010, and UPAS-120 showed consistent performance in rainfed conditions for multiple traits and across more than one year.

Kumar *et al.* (2021) assessed 28 genotypes of redgram for testing stability and and adaptability by using AMMI and GGE biplot method across different rainfed locations. Environment had the greatest influence on grain yield (56.8%), followed by the G x E interaction (27.6%) and genotype alone (18.6%). The analysis revealed two mega-environments and identified top-performing genotypes: ICPH 2740 (G15), TS 3R (G10), PRG 176 (G8), and ICPL 96058 (G22). ICPH 2740, PRG 176, and TS 3R performed best across all environments, while PRG 158 (G9), ICPL 87119 (G12), ICPL 20098 (G19), and ICPL 96058 (G22) indicated consistent adaptability over a range of conditions, with ICPH 2740 and PRG 176 being ideal candidates for general cultivation.

Ojwang *et al.* (2021) five pigeonpea genotypes across three different environments to assess genotype × environment interaction. The combined ANOVA over locations revealed highly significant G × E effects for traits such as seed yield, 100-seed weight, days to flowering, and days to maturity. In the AMMI analysis, the interaction principal components explained a large proportion of the variation in grain yield, with IPCA accounting for about 96.5% of the total variation. Genotypes MZ 2/9 and KAT 60/8 recorded low IPCA1 scores, indicating their stable performance and wide adaptability across environments. Among the test locations, Environment 3 (Kambiya Mawe) was identified as the most suitable site for genotype evaluation. In

terms of yield performance, KIONZA emerged as the top-performing genotype, while MZ 2/9 and KAT 60/8 were recognized for their stability and adaptability over a range of conditions.

Yohane *et al.* (2021) analyzed Grain yield stability and genotype × environment interaction in a set of 81 pigeonpea genotypes using both AMMI and GGE biplot approaches. The analysis showed that variation in grain yield was largely influenced by GEI, which contributed the highest proportion, followed by environment and genotype effects. Based on seasonal and locational differences, the test environments were classified into three distinct mega-environments. Both AMMI and GGE analyses consistently highlighted MWPLR 24, ICEAP 01155, MWPLR 14, TZA 5582, and MWPLR 4 as superior performers, combining high yield with stable performance across diverse conditions.

Kishore *et al.* (2022) assessed a set of 14 elite pigeonpea genotypes was evaluated to study genotype × environment crossover interactions using AMMI and GGE biplot stability models. From the GGE biplot analysis for yield, genotype WRG-437 emerged as the best performer in E3. In E2, WRGE-134 showed the highest performance, followed by WRGE-143 and WRGE-138. Meanwhile, in the other mega-environment, E1, WRGE-141 stood out as both high yielding and stable. Overall, the findings from both AMMI and GGE analyses consistently highlighted the differential performance of genotypes across environments. Genotypes WRGE-138, WRGE-134, WRGE-136, and WRGE-143 were identified as the most adaptable lines, exhibiting stable performance with minimal G×E interaction, and they can be considered promising candidates for boosting pigeonpea productivity.

Arega *et al.* (2023) investigated the yield of late-maturing pigeonpea genotypes was substantially influenced by genotype-environment interaction (GEI). The yield components themselves were also significantly impacted by GEI. The partitioning of the total sum of squares for genotype plus genotype × environment interaction (G + GE) indicated that the first two interaction principal components made substantial contributions, with IPCA1 and IPCA2 accounting for 29.72% and 34.86% of the variation, respectively. Across the evaluated environments, genotypes ICEAP 01499 and ICEAP 01489 stood out for their consistent performance, combining stability with relatively high yield.

Bomma *et al.* (2024) conducted an experiment with a total of 37 pigeonpea genotypes were evaluated to understand genotype × environment interaction for grain yield using GGE and AMMI biplot analyses. In addition, stability parameters such as AMMI stability value (ASV) and yield relative to environmental maximum (YREM) were applied to identify genotypes that combine high yield with stability.The results showed that the first two interaction principal components contributed substantially to the variation, with IPC1 explaining 40.6% and IPC2 accounting for 23.3%. Based on overall ranking, genotypes ICPL 20205, ICPL 20203, ICPL 19404, ICPL 19415, and ICPL 19405 emerged as top performers. Among all entries, ICPL 19405 stood out as the most promising, showing stable and high yield performance across locations. This genotype recorded an average grain yield of 1,469.30 kg/ha, along with a low ASV (3.82) and a favorable yield stability index (YSI = 13), making it a strong candidate for future evaluation and possible release.

Rao *et al.* (2024) An experiment was conducted using nine elite pigeonpea genotypes across different locations to evaluate their stability through AMMI and GGE biplot analyses. The findings revealed that the first two principal components explained a major share of the interaction variation, contributing 73.4% in AMMI and 80.50% in the GGE biplot. Both methods consistently identified WRGE-126 as a superior genotype, combining high productivity (1,733 kg ha^{-1}) with stable performance across environments. Among the test locations, Tandur emerged as the most favorable environment for evaluation, while Adilabad and Warangal were found to be highly representative and effective in discriminating among genotypes.

Aruselvi *et al.* (2025) evaluated 38 genotypes of red gram to estimate yield stability utilizing AMMI and GGE biplot model. The analysis revealed that both genotype and environment, along with their interaction, were key factors affecting grain yield in pigeonpea. The first two principal component axes (IPCA I and IPCA II) were statistically significant and together accounted for the full degrees of freedom of the interaction effect. Genotype ICPL 87 performed best in environment E3, while genotypes ICPL 88036-1, PA 477, and ICPL 149 were the top performers in environment E1. Environments E1 and E2 were similar to each other. Among the genotypes evaluated, ICPL 88036-1 and PA 477 produced high yields and displayed strong yield stability, making them suitable for planting across all three seasons. In

contrast, ICPL 87 and ICPL 149 gave high yields but were less stable. Genotypes ICPL 12337, ICPL 20325, CORG 9701, and ICPL 88034 were more stable but yielded less.

12.4 Molecular characterization using simple sequence repeats (SSRs)

Rajamani *et al.* (2020) assessed molecular diversity among nine pigeonpea varieties grown in Andhra Pradesh, using DNA fingerprinting with 50 polymorphic SSR markers. A total of 184 alleles were detected, averaging 3.68 alleles per marker. The marker CcM0481A produced the highest number of alleles (eight). Polymorphic Information Content (PIC) values ranged from 0.00 to 0.84, with a mean of 0.52. The neighbour-joining analysis yielded three clusters. Variety LRG-223 formed its own cluster (Cluster I), whereas the remaining eight varieties grouped in Cluster II, suggesting that LRG-223 has a distinct genetic background.

Sharma *et al.* (2020) evaluated 96 pigeonpea accessions using 44 simple sequence repeat (SSR) markers spread across all 11 chromosomes. Of these 44 markers, 33 were polymorphic, representing 75% polymorphism. Polymorphic information content (PIC) values varied from 0.26 for marker CCM 0183 to 0.78 for markers CCM 0402 and CCM 0721. Using the 112 alleles generated by the SSR markers, the 96 genotypes were divided into eight clusters. Clusters I and VII were the largest, each containing 22 genotypes; clusters III and IV were the smallest, with 2 genotypes each; clusters II, V, VI, and VIII contained 10, 15, 14, and 9 genotypes, respectively. Pusa 991 and ULA 11 were the most genetically distinct pair, showing the highest dissimilarity coefficient (32%), while AL 112A and AL 113A were the most similar, with the lowest dissimilarity coefficient (2%). These highly divergent genotypes could be useful in pigeonpea breeding programs to obtain desirable segregants.

Reddy *et al.* (2022) assessed 32 pigeonpea genotypes for molecular diversity, using 20 SSR markers. The 15 polymorphic markers yielded 43 alleles in total, averaging 2.15 alleles per marker, with CcM 0008 and CcM 1026 each producing the highest count of four alleles. Polymorphic Information Content (PIC) values ranged from 0.06 for PGM 16 to 0.64 for CcM 0008, with a mean of 0.19. A UPGMA-derived dendrogram separated the 32 genotypes into 10 clusters, the largest being Cluster I with 15 genotypes, followed by Cluster V with five. A neighbour-joining tree based on the weighted average of the dissimilarity matrix grouped the same 32 genotypes into eight

clusters, with Group II being the largest (10 genotypes). The analysis identified Kunnathur local, CRG 13–01, BSR 1, and Pillayakothur local as the most genetically distinct genotypes, possessing desirable grain and vegetable-type traits suitable for breeding. .

Yohane *et al.* (2022) evaluated 81 pigeonpea genotypes for genetic variability and population structure sourced from Malawi, Tanzania, and Kenya, using 4,122 SNP markers alongside morphological characteristics. Both the SNP markers and phenotypic traits indicated considerable genetic variation across the genotypes. Based on both marker systems, the genotypes were clearly grouped into three distinct clusters. The analysis revealed an average gene diversity of 0.14, while the polymorphic information content (PIC) was estimated at 0.11, suggesting a moderate level of genetic variation. Further, molecular variance analysis indicated that the majority of variation (97.3%) existed among individuals within populations, whereas only a small proportion (2.7%) was attributed to differences between populations.

Kumar *et al.* (2023) experimented with 30 pigeonpea genotypes for molecular characterisation. Genomic DNA from 30 pigeonpea genotypes was amplified using 20 SSR primers, yielding 46 total bands. Of these, 30 were polymorphic (65.21%) and 16 were monomorphic (34.82%). Among the primers used, CcM 2977 generated the maximum number of bands, of which two were polymorphic. Overall, the analysis showed that out of 20 primers, eight exhibited complete polymorphism (100%), whereas five were completely monomorphic. On average, each primer produced about 2.30 bands. Based on the combined marker data, the dendrogram grouped the genotypes into six distinct clusters, with five of these clusters each containing only a single genotype.Cluster analysis, performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), grouped the 30 genotypes into six main categories.

Rao *et al.* (2024) assessed 43 extra early genotypes to examine the genetic diversity using 41 HASSR markers. Out of that 1 produced distinct banding profiles, 11 exhibited monomorphic patterns, and 9 failed to amplify. Cluster analysis using these HASSR markers indicated that ICPL87119 was the most genetically distinct; this genotype was also found to be resistant to wilt. Based on similarity coefficients and clustering, ICPL87119, ICPL8403, CPL88014, ICPL161, ICPL87091 and CORG9701 were genetically more different from other genotypes

13. Table 1: Materials and Methods

13.1	Location	:	Pulses Research Station, Junagadh Agricultural University, Junagadh
13.2	Year and season of experiment a) For development of crosses b) For evaluation of experimental materials	 : :	 *Kharif* 2026-27 *Kharif* 2027-28
13.3	Crossing pattern	:	Half-diallel mating Design
13.4	No. of genotypes	:	37 [8 Parents + 28 Hybrids + 1 Standard Check (GT 111)]
13.5	No. of environments	:	Four dates of sowing
13.6	Experimental design	:	Randomized Block Design (RBD)
13.7	No. of replications	:	3 (Three)
13.8	Spacing	:	90 cm x 20 cm

13.9 Table 2: List of genotypes used for crossing

Sr. No.	Genotype	Souce
1	GJP 2304	PRS, Junagadh
2	GJP 2309	PRS, Junagadh
3	GJP 2505	PRS, Junagadh
4	GJP 2510	PRS, Junagadh
5	PA 2018-4	IARI, New Delhi
6	PA 2016	IARI, New Delhi
7	BRG-22-407	Bangalore
8	ICPL 22408	ICRISAT, Hyderabad

13.10 Observations to be recorded

Measurements will be obtained from five randomly selected competitive plants per genotype in each replication for most traits, whereas days to 50% flowering and days to maturity will be assessed on a plot-wise basis.

13.10.1 Plant height (cm)

At physiological maturity, the height from the soil surface to the top of the main stem will be measured and reported in centimetres**.**

13.10.2 Number of primary branches per plant

The number of pod-bearing branches on the main stem of each plant will be counted at physiological maturity.

13.10.3 Number of secondary branches per plant

The number of pod-bearing branches on the primary branches of a single plant will be recorded at the time of physiological maturity.

13.10.4 Days to 50 percent flowering

Days to 50% flowering will be noted as the time taken from sowing to when half of the plants in the plot have flowered.

13.10.5 Days to maturity

The number of days from the date of sowing to the date when about 75 per cent of pods in each genotype reached physiological maturity will be recorded.

13.10.6 Number of pods per plant

The total number of seed-bearing pods obtained from randomly selected plants at the time of maturity will be recorded.

13.10.7 Pod length (cm)

The length of randomly selected pods of five from each genotype will be taken and their mean value will be expressed in centimeters.

13.10.8 Number of seeds per pod

For each genotype, the number of seeds in five randomly chosen pods will be counted and their mean value will be expressed as seeds per pod.

13.10.9 Hundred seed weight (g)

One hundred matured and well dried seeds will be selected randomly and weighed using an electronic balance and expressed in grams.

13.10.10 Seed yield per plant (g)

Dry pods will be harvested from each selected plant and threshed separately. Seed weights will be recorded after thorough sun drying and expressed in grams.

13.10.11 Seed protein content (%)

By Folin-Lowry method (1951), the individual seed sample protein content will be estimated.

14. Statistical Analysis

The mean performance of parents and F_1 hybrids for each trait will be analyzed using the following statistical methods.

14.1 Analysis of variance for experimental design

The statistical analysis of variance for each trait will be undertaken using the Randomized Block Design approach in accordance with Panse and Sukhatme (1995).

14.2 Estimation of heterosis

It is more relevant to use data from better parents for heterobeltiosis as well as to verify variety for analysis of standard heterosis, sometimes referred to as economic heterosis, when estimating heterosis.

(A) Heterobeltiosis

According to Fonseca and Patterson (1968), heterobeltiosis is the deviation of F_1 from the better parent and expressed in percentage.

$$\text{Heterobeltiosis (\%)} = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Where,

$\overline{F_1}$ = Average performance of F_1

$\overline{BP}$ = Average value of better parent of respective cross combination

(B) Standard heterosis

It is superiority of F_1 over the standard commercial variety (GT-111, check) (Falconer and Mackay, 1996) and will be calculated as below:

$$\text{Standard heterosis (\%)} = \frac{\overline{F_1} - \overline{SC}}{\overline{SC}} \times 100$$

Where,

$\overline{F_1}$ = Average performance of F_1

$\overline{SC}$ = Average performance of the standard check commercial variety.

14.3 Analysis of variance for combining ability and gene action

The Griffing (1956) recommended that the half-diallel mating design will be used to perform the combining ability analysis.

14.4 Components of genetic variance

Numerical approach of Hayman (1954) will be used to workout components of genetic variation and other genetic ratios.

14.5 Analysis of variance for G x E interactions and phenotypic stability

The statistical analysis for genotype x environment interaction and phenotypic stability will be carried out as follows;

14.5.1 Eberhart and Russell model

It is a regression coefficient-based analysis, where the deviation from regression analysis is used to describe the stability of genotype and it is described by Eberhart and Russell (1966).

14.5.2 AMMI

It is a multivariate analysis combining ANOVA and PCA for estimating effects of Genotype x Environment interaction on stability of genotypes, and is described by Gauch and Zobel (1992). It helps to identify the genotypes that perform stably across diverse environments.

14.6 Molecular characterization using SSR markers

Genomic DNA will be isolated and purified using the Cetyl Trimethyl Ammonium Bromide (CTAB) protocol, with any necessary adjustments, following the methodology established by Doyle and Doyle (1990). High-purity DNA samples will then be utilized for the Polymerase Chain Reaction (PCR)
amplification of specific molecular markers. PCR amplification and subsequent gel electrophoresis will be performed employing Simple Sequence Repeat (SSR) markers. The resulting PCR product bands will be scored, and the marker data will be analyzed using appropriate bioinformatics tools.

Observations regarding each molecular marker will be scored individually, and comprehensive statistical summaries of the molecular marker data will be computed with suitable software.

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